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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
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ART UNIT	PAPER NUMBER
1636	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/692,553	COURT ET AL.	
	Examiner	Art Unit	
	JENNIFER DUNSTON	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 November 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3-10,12,13,22,23 and 27-29* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-10,12,13,22,23 and 27-29* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *23 August 2006* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 11/12/2008 has been entered.

Receipt is acknowledged of an amendment, filed 11/12/2008, in which claims 14-21 and 24-26 were canceled, claim 1 was amended, and claims 27-29 were newly added. Claims 1, 3-10, 12, 13, 22, 23 and 27-29 are pending.

Election/Restrictions

Applicant elected of Group I without traverse in the reply filed on 12/5/2005. Currently, claims 1, 3-10, 12, 13, 22, 23 and 27-29 are under consideration.

Priority

Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged. Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 120 as follows:

The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application). The disclosure of the invention in the parent application and in the later-

filed application must be sufficient to comply with the requirements of the first paragraph of 35 U.S.C. 112. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994).

The disclosures of the prior-filed applications, International Application No. PCT/US01/25507 and Provisional Application Nos. 60/225,164 and 60/271,632, fail to provide adequate support or enablement in the manner provided by the first paragraph of 35 U.S.C. 112 for one or more claims of this application. The prior-filed application numbers do not provide literal or inherent support for the claimed method steps of claims 1, 3-10, 12, 13, 22, 23 and 27-29. While the prior-filed applications suggest that the disclosed method of homologous recombination may be used to construct complex conditional targeting vectors, the specifications do not set forth the claimed method steps. For example, the prior-filed applications do not provide adequate written description for the method step of using homologous recombination to insert a nucleic acid encoding a selectable marker flanked by a pair of second recombining sites and a first recombining site into a second site into the gene in a bacterial artificial chromosome. The prior-filed applications do not teach how to use the disclosed recombination system to make a vector for the conditional knockout of a gene, where two first recombining sites remain in a gene and recombination of the two first sites produces a nucleic acid sequence that cannot be transcribed to produce a functional protein.

Claims 1, 3-10, 12, 13, 22, 23 and 27-29 have an effective filing date of 2/12/2003.

Claim Objections

Claim 28 is objected to because of the following informalities: the term "EM7" is misspelled at line 12, and the word "chromosome" is misspelled at line 15 of the claim. Appropriate correction is required.

Claim 6 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. Claim 6 depends from claim 1, which limits the cell in which the vector is generated to a bacterial cell. Claim 6 encompasses the use of any host cell and thus is broader in scope than claim 1.

Claim 10 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. Claim 10 indirectly depends from claim 1, which limits the cell in which the vector is generated to a bacterial cell. Claim 10 limits the cell to a bacterial cell and thus does not further limit claim 1 or 7.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 3-10, 12, 13, 22, 23, 27 and 28 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lee et al (Genomics, Vol. 73, pages 56-65, 2001, cited in a prior action; see the entire reference) in view of Economides et al (US Patent No. 6,586,251 B2; see the entire reference) and Meyers et al (Nature Genetics, Vol. 18, pages 136-141, February 1998; see the entire reference). This is a new rejection.

Lee et al teach an *E. coli*-based system for bacterial artificial chromosome (BAC) engineering (e.g., Title; page 57, left column, 1st full paragraph). Lee et al teach the use of *E. coli* containing a defective λ prophage comprising *gam*, *exo* and *bet* (encoding Gam, Exo and Beta, respectively) under the control of the P_L promoter, which is repressed by the temperature-sensitive repressor CI857 at 32 °C and derepressed at 42 °C, and containing either cre or flpe under the control of an inducible promoter (e.g., Figure 1). Lee et al teach that the λ prophage system appears to be 50-100 fold more efficient than the plasmid based λ recombination system or the RecET system (e.g., page 56, right column, 1st paragraph). Lee et al teach that the recombination system facilitates the generation of complicated conditional targeting vectors (e.g., page 64, right column, 2nd paragraph). Lee et al teach the introduction of a selectable marker flanked by LoxP or FRT sites into an intron of a gene using the bacterial homologous recombination system, and removal of the selectable marker by inducing transient expression of Cre or Flpe recombinase, respectively (e.g., page 64, right column, 2nd paragraph). Lee et al exemplify a recombination cassette comprising a kanamycin resistance gene flanked by FRT sites (FRT-kan-FRT; e.g., page 57, left column, last full paragraph). Lee et al teach that the bacterial homologous recombination system and reversible expression of Cre or Flpe

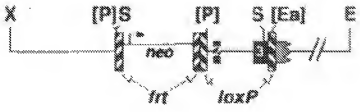
recombinase greatly speeds up the process of making conditional targeting vectors (e.g., page 64, right column, 2nd paragraph).

Lee et al do not explicitly teach using homologous recombination to insert a nucleic acid sequence encoding a second selectable marker flanked by a pair of second recombining sites into a second site in the gene; and excising the nucleic acid encoding the second selectable marker with a second recombinase for the second recombining sites, where recombination of the remaining recombining sites produces a nucleic acid sequence that cannot be transcribed to produce a functional protein.

Economides et al teach a method for generating large DNA targeting vectors for eukaryotic cells, termed LTVECs (e.g., Abstract; column 3, lines 12-55). Economides et al specifically teach generating an LTVEC for making a conditional allele of a gene (e.g., column 3, line 52; column 10, lines 32-39). Economides et al teach that the problem of engineering precise modifications into very large genomic fragments, such as those cloned into BAC libraries, has largely been solved through the use of homologous recombination in bacteria (e.g., column 2, lines 42-52). Economides et al specifically teach using bacterial homologous recombination to engineer a desired modification within a large genomic fragment, such as a BAC, thereby crating the LTVEC (e.g., column 3, lines 12-22; column 7, lines 53-59; column 8, lines 16-28). To construct a LTVEC, Economides et al teach (i) obtaining a large genomic clone containing the gene(s) or chromosomal locus (loci) of interest, where the clone is a BAC; and (ii) appending homology boxes to a modification cassette and using bacterial homologous recombination to generate the LTVEC (e.g., column 9, line 12 to column 10, line 14). For LTVEC to make conditional alleles, Economides et al teach the introduction of LoxP sites

flanking the region to be excised by Cre recombinase, or FRT sites flanking the region to be excised by Flp recombinase (e.g., column 10, lines 32-39).

Meyers et al teach that the repertoire of allele types that can be generated in mice has been expanded by the development of methods for making gene alteration in mice conditional upon recombination mediated by a site-specific DNA recombinase such as Cre or Flp (e.g., page 136, left column, 2nd paragraph). Meyers et al teach that recombinase-modifiable alleles contain LoxP or FRT sites, the recognition sequences for Cre or Flp, respectively; in the progeny of a cross between mice carrying a conditional allele and transgenic mice carrying the appropriate recombinase gene, recombination between two directly repeated LoxP or FRT sites resolves them to a single site, thereby deleting the gene sequence that lie between them (e.g., page 136, left column, 2nd paragraph). When the targeted locus contains two LoxP sites and two FRT sites, it is modifiable by a Cre, Flp or both (e.g., page 136, left column, 3rd paragraph). Meyers et al teach a targeting vector to produce an allelic series for the mouse *Fgf8* gene. The vector contains *Fgf8* genomic DNA where one LoxP site was inserted in the intron upstream of exon 2, a second LoxP site was inserted in the 3'-untranslated region (UTR) in order to "flox" (flank with LoxP sites) the *Fgf8* coding sequence in exons 2 and 3; and a neomycin-resistance expression cassette flanked by FRT sites (flrted) was inserted immediately upstream of the 5' LoxP site (e.g., page 136, right column, 1st full paragraph; Figure 1a). Meyers et al teach that the LOXP sites and flrted neo cassette, comprising the promoter and 3'-UTR of the mouse Pgk1 gene, interrupts the *Fgf8* coding sequence (e.g., page 136, right column, 1st full paragraph). The targeting vector is shown in Figure 1a, which is reproduced below:



Thus, the two LoxP sites present in the vector flank the region to be conditionally deleted to form a nucleic acid sequence that cannot be transcribed to produce a functional Fgf8 protein (e.g., Figures 1d and 1f).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the teachings of Lee et al to include the second LoxP or FRT site taught by Economides et al such that the sites flank exons to be deleted as taught by Meyers et al because Lee et al, Economides et al, and Meyers et al teach it is within the ordinary skill in the art to use LoxP and/or FRT sites in the construction of conditional targeting vectors. Lee et al specifically suggest the use of bacterial homologous recombination to insert a nucleic acid sequence encoding a first selectable marker and flanked by a first recombining sites into a first site in the intron of a gene, followed by excising the nucleic acid encoding the selectable marker with a first recombinase specific for the first recombining sites. These steps result in the placement of a first LoxP site in the intron of a gene in a BAC. Economides et al teach the use of BAC vectors to construct conditional allele targeting vectors, where the site-specific recombination sites (LoxP or FRT) flank the region to be excised by the recombinase. Myers et al teach the use of site specific recombination sites to flank exons, which will result in the conditional deletion of a nucleic acid sequence such that a functional protein cannot be produced. Thus, the art teaches where to place the LoxP or FRT sites in the gene targeting vector, and to use BACs modified by

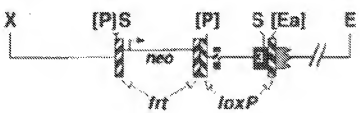
bacterial homologous recombination as conditional targeting vectors. To generate a conditional targeting vector suggested by Lee et al, it would have been obvious to one of skill in the art to include the second LoxP or FRT site flanking an exon by repeating the steps of inserting a selectable marker flanked by recombining sites into a second site in the gene, followed by excising the nucleic acid encoding the selectable marker with a recombinase. Since the references teach the use of LoxP or FRT sites, and the sites serve the same function, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have LoxP as the first and second recombining sites, or FRT as the first and second recombining sites. Because both Lee et al and Economides et al teach the use of a kanamycin resistance gene, it would have been obvious to one of ordinary skill in the art to replace the kanamycin resistance gene of Lee et al with the PGK-EM7-neomycin resistance gene cassette, which also provides kanamycin resistance in bacterial cells, in order to achieve the predictable result of providing a recombination cassette for bacterial homologous recombination that can be positively selected by kanamycin resistance.

One would have been motivated to make such a modification in order to receive the expected benefit of providing a vector for conditional targeting as taught by Economides et al with the arrangement of recombination sites taught by Meyers et al using a more efficient bacterial homologous recombination system for BAC modification as taught by Lee et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claim 28 requires the steps of (i) using homologous recombination in a bacterial cell to

insert a nucleic acid encoding a first selectable marker operably linked to a PGK-EM7 promoter flanked by a pair of LoxP sites into a first site in a gene in a bacterial artificial chromosome, wherein a vector comprises the bacterial artificial chromosome, and wherein the bacterial cell comprises a de-repressible promoter operably linked to a nucleic acid encoding Beta and Exo, and wherein using homologous recombination comprises de-repressing the de-repressible promoter, thereby inducing the expression of Beta and Exo; (ii) excising the nucleic acid encoding the selectable marker with Cre recombinase, resulting in a single LoxP site remaining in the bacterial artificial chromosome; (iii) using homologous recombination in the bacterial cell to insert a nucleic acid molecule comprising a FRT site 3' and 5' of a nucleic acid encoding a second selectable marker operably linked to PGK-EM7 promoter, wherein the nucleic acid molecule further comprises a LoxP site 3' of the 3' FRT site; and (iv) excising the nucleic acid encoding the second selectable marker with FLP, resulting in a second LoxP site and a FRT site remaining in the bacterial artificial chromosome, thereby generating the vector for conditional knockout of a gene. Lee et al teach the step of inserting a nucleic acid encoding a selectable marker flanked by LoxP sites into a gene (first site) in a bacterial artificial chromosome using bacterial homologous recombination, where the bacterial cell for recombination comprises a de-repressible promoter operably linked to a nucleic acid encoding Beta and Exo, and where using homologous recombination comprises de-repressing the de-repressible promoter, thereby inducing the expression of Beta and Exo. Further, Lee et al teach excising the selectable marker by transiently expressing Cre recombinase. Lee et al do not specifically teach the use of a PGK-EM7 promoter flanked by a pair of LoxP sites. However, both Lee et al and Economides et al teach the use of a kanamycin resistance marker flanked by site-specific recombination sites, and

Economides et al teach the use of the PGK-EM7 promoter. Thus, it would have been obvious to one of ordinary skill in the art at the time the invention was made to include the PGK-EM7 promoter to drive the expression of the selectable marker in order to achieve the predictable result of expressing the selectable marker in the bacteria in which homologous recombination occurs. Further, Lee et al do not teach using homologous recombination in the bacterial cell to insert a nucleic acid molecule comprising a FRT site 3' and 5' of a nucleic acid encoding a second selectable marker operably linked to PGK-EM7 promoter, wherein the nucleic acid molecule further comprises a LoxP site 3' of the 3' FRT site; and excising the nucleic acid encoding the second selectable marker with FLP, resulting in a second LoxP site and a FRT site remaining in the bacterial artificial chromosome, thereby generating the vector for conditional knockout of a gene. However, Meyers et al teach a targeting vector of the following structure:



This vector has a single LoxP site inserted into the 3'UTR of the gene. Further, it contains a neomycin reporter flanked by FRT sites and further comprising a LoxP site 3' of the 3' FRT site. It would have been obvious to one of ordinary skill in the art to make this structure using the bacterial recombination system of Lee et al, where the neo cassette comprises the PGK-EM7-neo cassette of Economides et al, because both Meyers et al and Economides et al teach the use of a neomycin cassette, and Lee et al teaches the use of a kanamycin cassette. The neomycin resistance gene provides kanamycin resistance in bacterial cells. Thus, one would use the PGK-

EM7-neo cassette in order to achieve the predictable result of providing a kanamycin resistance cassette capable of being expressed in the bacteria used for homologous recombination. It would have been obvious to one of ordinary skill in the art to insert the FRT-neo-FRT-LoxP sequence shown in the targeting vector of Meyers et al as a single recombination event using the bacterial recombination system of Lee et al, because Lee et al teach the system for inserting a selectable marker flanked by recombination sites, and the LoxP site of Meyers et al is immediately 3' to the FRT site. One would recognize that this portion of the construct could be inserted in a single step to save time. Furthermore, Meyers et al teach that the neomycin resistance gene can be removed by FLP recombinase prior to the production of a mouse line in order to prevent the creation of a hypomorphic allele (e.g., paragraph bridging pages 136-137). It would have been obvious to one of skill in the art to remove the neomycin resistance gene in order to receive the expected benefit of not affecting the level of transcript prior to the deletion of the portion of the gene flanked by LoxP sites in the targeted mouse.

Claim 29 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lee et al (Genomics, Vol. 73, pages 56-65, 2001, cited in a prior action; see the entire reference) in view of Economides et al (US Patent No. 6,586,251 B2; see the entire reference) and Meyers et al (Nature Genetics, Vol. 18, pages 136-141, February 1998; see the entire reference) as applied to claims 1, 3-10, 12, 13, 22, 23 and 27 above, and further in view of Stewart et al (US Patent No. 6,355,412 B1, cited in a prior action; see the entire reference). This is a new rejection.

The combined teachings of Lee et al, Economides et al and Meyers et al are described above and applied as before.

Lee et al, Economides et al and Meyers et al do not teach the length of the sequence homologous to the site in the bacterial artificial chromosome.

Stewart et al teach a method of performing homologous recombination in a host cell, comprising introducing a nucleic acid sequence encoding RecE/T or Redα/β recombinase (i.e. Lambda Exo and Beta) into a host cell, introducing a polynucleotide comprising a nucleotide sequence homologous to the nucleotide sequence of interest into the host cell, activating the expression of RecE/T, and selecting a cell from the population in which homologous recombination has occurred (e.g. column 28, lines 10-50; column 29, lines 9-35; column 28, line 51 to column 29, line 8; columns 25-27; paragraph bridging columns 37-38). Further, Stewart et al teach the use of Gam in addition to Exo and Beta or RecE/T (e.g. column 25, lines 5-28; Example 1). Stewart et al teach that a variety of host-vector systems may be utilized to introduce and express the protein-coding sequence of RecE/T or Redα/β, including prokaryotic and eukaryotic cells such as bacterial, yeast, plant, rodent, mice, human, insect or mammalian cells (e.g. column 28, lines 10-40). With respect to regulatory controls, Stewart et al teach that a range of different expression levels and a variety of regulatory sequences are known in the art and the ability to generate a wide range of expression is advantageous for utilizing the method (e.g. column 25, lines 5-44; column 24, line 50 to column 25, line 3). Stewart et al teach that the expression can be regulated by the P_L promoter of phage λ and the inducible lambda repressor CI_{857} (e.g. column 26, lines 1-27). Stewart et al teach that the nucleotide sequence of interest may be extrachromosomal and located on a bacterial artificial chromosome (e.g. column 20, lines 37-57; paragraph bridging columns 28-29). Stewart et al teach that homology arms are required for recombination and are two short regions of double-stranded DNA homologous to

the sequence of the target DNA of interest (e.g., paragraph bridging columns 19-20). Stewart et al teach that the homology arms contain approximately 22 to 100 base pairs or more of continuous identity to a double-stranded region flanking the DNA of interest, which results in 44-200 bp of sequence homologous to the target site (e.g., paragraph bridging columns 19-20). Stewart et al teach that the lambda recombinases can be used to achieve high-efficiency targeted cloning (e.g. column 11, lines 3-47). Moreover, Stewart et al teach that when the recombination method is used in combination with site-specific recombination sites, the site-specific recombinase, which recognizes the sites is under the control of an inducible promoter such that upon induction of recombination expression, recombination between the site-specific recombination sites occurs (e.g., Figure 4).

Because Lee et al and Stewart et al teach the use of bacterial homologous recombination mediated by λ Beta, Exo and Gam, and Stewart et al teach the length of homology required for bacterial recombination mediated by λ Beta, Exo and Gam, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use regions of homology of 22-100 bp on each end of the sequence to be inserted in order to achieve the predictable result of providing regions of homology sufficient to promote bacterial homologous recombination.

Response to Arguments - 35 USC § 103

The rejection of claims 24-26 under 35 U.S.C. 103(a) as being unpatentable over Casanova et al in view of Lee et al is moot in view of Applicant's cancellation of the claims in the reply filed 11/12/2008.

The rejection of claims 24-26 under 35 U.S.C. 103(a) as being unpatentable over Casanova et al in view of Stewart et al is moot in view of Applicant's cancellation of the claims in the reply filed 11/12/2008.

Applicant's arguments, see pages 6-9, filed 11/12/2008, with respect to the rejection of claims 1, 3-10, 12, 13, 22 and 23 under 35 U.S.C. 103(a) as being unpatentable over Rajewsky et al in view of Lee et al have been fully considered and are persuasive. The previous rejection of claims 1, 3-10, 12, 13, 22 and 23 has been withdrawn.

Applicant's arguments, see pages 10-11, filed 11/12/2008, with respect to the rejection of claims 1, 3-10, 12, 13, 22 and 23 under 35 U.S.C. 103(a) as being unpatentable over Rajewsky et al in view of Muyrers et al and Stewart et al have been fully considered and are persuasive. The previous rejection of claims 1, 3-10, 12, 13, 22 and 23 has been withdrawn.

Applicant's arguments filed 11/12/2008 have been fully considered as they apply to the new rejections presented above but they are not persuasive.

At the paragraph bridging pages 8-9, the response asserts that Lee et al do not suggest, or render obvious inserting a second nucleic acid encoding a selectable marker flanked by a pair of second recombining sites into a second site in the gene. Further, the response asserts that Lee et al do not suggest, nor render obvious, excising the nucleic acid encoding the selectable marker with a second recombinase specific for the second recombining sites, wherein two first recombining sites remain in the gene following excision of the nucleic acid encoding the selectable marker, and wherein recombination of the first two recombining sites produces a nucleic acid sequence that cannot be transcribed to produce a functional protein. Specifically, at

page 9, 1st paragraph, the response asserts that Lee et al do not teach how to produce a nucleic acid sequence that cannot be transcribed into a functional protein.

These arguments are not found persuasive. Lee et al teach the application of the *E. coli*-based homologous recombination system for engineering BACs for the generation of complicated conditional targeting vectors (e.g., Title; page 64, right column, 2nd full paragraph). Lee et al do not explicitly teach the introduction of a second nucleic acid encoding a selectable marker flanked by a pair of second recombining sites; however, the prior art teaches that conditional targeting vectors must contain recombining sites that flank the region to be excised (Economides et al. US Patent No. 6,586,251 B2, e.g., column 10, lines 32-39; Meyers et al. Nature Genetics, Vol. 18, pages 136-141, February 1998, e.g., pages 136-137, Production of an *Fgf8* allelogenic mouse line, and Figure 1). Thus, Lee et al suggest the use of the bacterial recombination system for the insertion of a selectable marker flanked by recombining sites into a BAC for the construction of a conditional vector, and one skilled in the art would have recognized that a second site would need to be inserted to complete the construction of the conditional targeting vectors. Thus, one would have repeated the process taught by Lee et al to insert the second site. Only claim 28 requires two first recombining sites remain in the gene following excision of the nucleic acid encoding the second selectable marker. This structure comprising two second recombining sites flanking a selectable marker and a first recombining site 3' of the 3' second recombining site is taught by Meyers et al. Thus Lee et al is not relied upon for this particular limitation. Furthermore, Meyers et al clearly demonstrate that targeting vectors comprising site-specific recombination sites flanking exons of a gene will result in the formation of a nucleic acid sequence that cannot be transcribed to produce a functional protein

(e.g., page 136, right column, last ful6l paragraph; page 137, An *Fgf8* null allele via Cre-mediated recombination; Figure 1).

At page 9, 2nd full paragraph the response asserts that Lee et al do not suggest: (1) the use of two selectable markers each flanked by recombining sites; (2) the use of homologous recombination to make vectors for conditional knockouts; or (3) specific steps in any method that would lead to a vector for a conditional knockout.

These arguments are not found persuasive. With respect to point (2), Lee et al specifically teach the use of the *E. coli*-based recombination system for use in the construction of vectors for conditional knockouts (see page 64, right column, 2nd full paragraph). With respect to points (1) and (2), Lee et al teach the steps of introducing a selectable marker flanked with LoxP or FRT sites into an intron of a gene, and then removing the selectable marker by transient Cre of Flpe expression, leaving behind a solo LoxP or FRT site in the intron (e.g., page 64, right column, 2nd full paragraph). Combining these steps with the teachings of Economides et al and Meyers et al would result in the specific method steps claimed for the reasons set forth above. Briefly, conditional targeting vectors are known in the art to require two recombination sites flanking the region to be deleted. Lee et al teach the use of the method for conditional targeting vectors, yet only one site is introduced into the vector. In view of the teachings of Economides et al and Meyers et al, one would realize that the process of Lee et al would be repeated to introduce the second recombination site.

At page 9, the response asserts that the Declaration of Dr. Liu, filed 3/7/2008 discloses a large number of experiments required to devise the claimed methods is a secondary consideration. These experiments do not demonstrate that the references being relied on would

not enable a skilled artisan to produce the different compounds as claimed. The experiments do not demonstrate any unexpected results. The number of experiments does not provide evidence sufficient to overcome the rejections presented above.

Response to Amendment – Declaration of Pentao Liu and E-Chiang Lee

The declaration under 37 CFR 1.132 filed 11/12/2008 is sufficient to overcome the rejection of claims 1, 3-10, 12, 13, 22 and 23 based upon the Rajewsky et al reference applied under 35 U.S.C. 103(a).

The claims now require the generation of the vector in a bacterial cell. Rajewsky et al teach the removal of the selectable marker flanked by the second recombination sites in ES cells.

The declaration under 37 CFR 1.132 filed 11/12/2008 is insufficient to overcome the new rejection of claims 1, 3-10, 12, 13, 22, 23 and 27-29 based upon Lee et al as set forth above.

The declaration discusses experiments disclosed in Lee et al that are directed to the construction of a mouse for functional expression of Cre protein. The declaration does not discuss the portions of the Lee et al reference cited in the above rejections. Specifically, the teachings directed to the construction of conditional targeting vectors are not discussed by the declaration. A reference is good for all that it teaches. Lee et al teaches the use of the disclosed *E. coli*-based recombination system for the construction of conditional targeting vectors (e.g., page 64, right column, 2nd paragraph).

The declaration notes that Lee et al do not use a second set of recombining sites flanking a second selectable marker. These limitations are met by the combined teachings of Lee et al, Economides et al, and Meyers et al as set forth above.

The response asserts that the present methods provide a rapid and efficient method for generating conditional targeting vectors, because it uses recombineering rather than restriction enzymes and DNA ligases for vector construction. Evidence of the efficiency of the disclosed recombineering system as compared to the use of restriction enzymes is not effective to overcome the above rejections. Lee et al and Economides et al teach the use of bacterial homologous recombination systems to construct conditional targeting vectors. Lee et al teach that the disclosed *E. coli*-based recombination system that makes use of a defective λ prophage is 50- to 100-fold more efficient than λ recombination functions expressed from a plasmid or the RecET system.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is 571-272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Low can be reached at 571-272-0951. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR

system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Jennifer Dunston, Ph.D.
Examiner
Art Unit 1636

/JD/

/ Christopher S. F. Low /
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